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The Mechanism of the Vasodilator Effect of Adrenaline

I. Effect on Skeletal Muscle Vessels

By

LENNART LUNDHOLM

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Ever since the discovery of adrenaline, interest has been focussed on its effect upon the circulation. Adrenaline has been chiefly regarded as a vasoconstrictor and hypertensor, even though Moore & Purington (1900), five years after its discovery, demonstrated that in low doses it had a depressor effect. Cannon & Lyman (1913) and Hoskins, Gunning & Berry (1916) found an increased blood flow in skeletal muscle coincident with the fall of blood pressure, indicating that adrenaline dilated the skeletal muscle vessels. However, adrenaline regularly had a depressor effect only when certain anesthetics were used; namely, ether, chloroform and urethane, the effect being weaker after chloralose (MacDonald & Schlapp, 1926; Vincent & Curtis, 1927). After anesthetization with barbiturates, and likewise in decerebrated and unanesthetized animals, the depressor effect was either weak or absent (Berry, 1917; Gruber, 1928, 1929; Dragstedt, 1928; Herwick, Linegar & Koppanyi, 1938). Moreover, it was found regularly only in dogs or cats. In view of these findings, the vasoconstrictor and hypertensor effect of adrenaline was long regarded as its principal physiologic and pharmacologic actions, and there was a tendency to consider the vasodilator effect as a phenomenon occurring only under certain artificial conditions.

Experiments on human subjects have shown, however, that the vasodilator effect is not merely a phenomenon produced in anesthetized animals; it is probably the most conspicuous of the effects on the circulation when adrenaline is administered in therapeutic doses or when present in the amounts secreted from the adrenals under physiologic conditions. Euler & Liljestrand (1927) demonstrated in man that a subcutaneous injection of 0.7 mg adrenaline increased the cardiac output by up to 100 per cent. The systolic blood pressure rose but the diastolic pressure

fell, so that the mean blood pressure was scarcely changed. These experiments made clear that adrenaline acted essentially by reducing the peripheral resistance, which conclusion was subsequently verified by STARR, GAMBLE, MARGOLIS, DONAL, JOSEPH and EAGLE (1937) and GOLDENBERG, PINES, BALDWIN, GREENE and ROH (1948). BARCROFT and his associates (for review, *vide* BARCROFT & SWAN, 1953), in a series of beautiful experiments on human subjects, demonstrated that the peripheral resistance regularly fell in the skeletal muscles both after intravenous and after intra-arterial injection of adrenaline. Lastly, CELANDER (1954), in experiments on cats, found that both in direct stimulation of the splanchnic nerves and after reflex activation of the adrenal medulla the most pronounced effect on the circulation lay in dilatation of the skeletal muscle vessels.

This modified view of the circulatory response to adrenaline enhances interest in the mechanism of the latter's vasodilator action. There is no generally accepted explanation of this action. In the present and subsequent papers, facts will be presented which suggest that the vasodilator effect of adrenaline is a result of its ability to induce lactic acid production in striated and smooth muscle. Moreover, light will be thrown on some of those factors due to which the vasodilator effect of adrenaline has been demonstrable only under certain experimental conditions.

Previously recognized facts that seem important to our understanding of this vasodilator effect are the following. The effect in question is — unless adrenergic blocking drugs have been administered — localized to the skeletal muscle vessels (HARTMAN, 1915; HOSKINS, GUNNING & BERRY, 1916; DALE & RICHARDS, 1918; GRUBER, 1928; REIN & SCHNEIDER, 1930; CLARK, 1934; BURN, 1938; GRANT & PEARSON, 1938; ALLAN, BARCROFT & EDHOLM, 1946; GRIFFITH, LOCKWOOD & LOOMIS, 1946; FOLKOW, FROST & UVNÄS, 1948; GOTTSTEIN, HILLE & OBERDORF, 1955; and others; for a more exhaustive bibliography — 42 references — *vide* DÖRNER, 1953), and the hepatic vessels (BRADLEY, 1946; BEARN, BILLING & SHERLOCH, 1951). The coronary vessels too were dilated by adrenaline, and this, indeed, after such small doses as to have no stimulating effect on the heart muscle (ECKENHOFF, HAFKENSCHIEL & LANDMESSER, 1947; WÈGRIA, 1951). As regards the skeletal muscle vessels the dilatation was even observed after total denervation (DALE & RICHARDS, 1918; WHELAN, 1952), and it could also be demon-

strated in isolated, perfused hind legs of cats (DALE & RICHARDS, 1927). Vasodilatation in the muscles persisted longer after continuous intravenous infusion of adrenaline than after intra-arterial infusion (DUFF & SWAN, 1951; WHELAN, 1952). WHELAN assumed this to imply that adrenaline liberated, in the organism, a substance having vasodilator characteristics. After administration of alkali the vasodilator effect of adrenaline in muscle was inhibited (MCDOWELL, 1941).

Lastly, there is the seemingly paradoxical circumstance that adrenaline in low concentrations dilates the muscle vessels, but in higher concentrations it constricts not only skeletal muscle vessels but most of the others too.

An acceptable theory of the vasodilator effect of adrenaline must, accordingly, explain why only some vascular areas are dilated; in what way various anesthetics influence the dilatation; why alkalization may weaken the vasodilator effect; the difference in action between intravenous and intra-arterial infusion of adrenaline; and, finally, how a change in the concentration of adrenaline can convert a dilator into a constrictor effect.

The manifold effects of adrenaline include its ability to stimulate the lactic acid production in skeletal muscles (CORI, FISHER & CORI, 1935; GRIFFITH, LOCKWOOD & LOOMIS, 1946), and the heart (GOTTDENCKER & MARCHI, 1937). Lactic acid is discharged into the blood and oxidized and resynthesized to glycogen in the liver (for review, *vide* CORI, 1931). Since lactic acid has a vasodilator action (GASKELL, 1880; KELLER, LOESER & REIN, 1930; FLEISCH & SIBUL, 1933; DOMINI & REIN, 1942) it appears to be a curious coincidence that the vasodilator effect of adrenaline was localized precisely to those vascular areas where it induced lactic acid production or where the formed lactic acid was oxidized. Earlier experiments had shown, moreover, that chloralose inhibited the lactic acid producing effect of adrenaline (LUNDHOLM, 1949). Alkalization of the blood should neutralize and prevent the action of formed lactic acid. It seemed possible, lastly, to explain the diametrically opposite effects, on skeletal muscle, of adrenaline in low and in high concentrations by assuming the vasodilator action to be induced by lactic acid production in skeletal muscle cells and the vasoconstrictor action by a direct effect on the smooth muscle layer of skeletal muscle vessels. — The threshold dose for the lactic acid producing effect of adrenaline is lower than that for the vasoconstrictor action (CORI, FISHER & CORI,

1935; GRIFFITH, LOCKWOOD & LOOMIS, 1946). — There was, accordingly, a good deal of evidence pointing to a connection between the vasodilator and the lactic acid producing effects of adrenaline.

In experimental testing of the hypothesis that the vasodilator effect resulted from adrenaline's ability to produce lactic acid, it was found appropriate to use varying methods. Earlier workers had demonstrated the vasodilator effect of adrenaline in the following ways. (1) By an increase of the blood flow in some vascular areas at a constant blood or perfusion pressure; adrenaline was administered intravenously or intra-arterially. (2) By a fall of blood pressure after intravenous injection of adrenaline. (3) By an increase in the cardiac output when the mean blood pressure was unchanged or only very slightly elevated; adrenaline was administered intravenously or subcutaneously. (4) By a reversal of the hypertensor or vasoconstrictor effect after administration of adrenergic blocking drugs.

It cannot be assumed, *a priori*, that the cause, localization and duration of the vasodilatation will be identical under these varying experimental conditions. Nor, indeed, is this the case. Intravenous infusion of adrenaline, as mentioned above, caused a more prolonged dilatation of skeletal muscle vessels than did intra-arterial infusion. Adrenaline dilated the intestinal vessels after administration of ergotamine, but in untreated animals it constricted them in all active concentrations (CLARK, 1934; ROTHLIN, 1945). In view of these circumstances, it was desirable to investigate any possible connection between the vasodilator and the lactic acid producing effect of adrenaline with all the methods enumerated above.

This first part will be concerned with the association between vasodilatation and lactic acid production in the skeletal muscle after intra-arterial infusion of adrenaline. The experimental conditions in this series were particularly favorable for a direct comparison of the vasodilator and the lactic acid producing effects, since it was possible both to determine the lactic acid production quantitatively with satisfactory accuracy and to investigate selectively the peripheral vasodilator action of lactic acid without interference from its stimulating effect on the vasomotor center. Subsequent papers will deal with the connection between the hypotensor effect of adrenaline and its lactic acid producing capacity in anesthetized cats, as well as the action

of adrenaline and lactic acid on the cardiac output of unanesthetized rabbits. Lastly, the final paper will treat the relation between adrenaline reversal after adrenergic blocking drugs and the lactic acid production.

The vasodilator effect of adrenaline in skeletal muscle has been a somewhat capricious phenomenon which not all workers have been able to demonstrate. — The first aim was, therefore, to establish experimental conditions under which that effect would be regularly demonstrable. Moreover, the vasodilator effect had to be of adequate duration, since if lactic acid was assumed to be produced only in connection with vasodilatation, the production might be too low for determination with sufficient accuracy by the methods of assay employed. The duration of vasodilatation as well as the lactic acid production were found to be greatly dependent on the anesthetic employed (see Part II). Of the anesthetics tried, ether proved to be the one under which the vasodilatation was most evident. Under chloralose as well as evipan anesthesia the vasodilator effect of intra-arterial infusion of adrenaline was initially distinct, but of comparatively short duration. Under urethane anesthesia the vasodilatation was almost as pronounced and prolonged as in the case of ether.

Methods

Cats weighing 2.5—5 kg were taken for the experiments. In small cats the blood loss associated with the taking of samples was too great and preparation of the animals was technically very difficult. — The experiments were performed under ether anesthesia, administered as follows. A Y-cannula was inserted into the trachea. One of its branches was connected, via a rubber tube, to a pump that pumped air through a flask containing ether. The arrangement, however, enabled a variable portion of the air to by-pass the ether flask, so that the ether content of the inspired air could be adjusted as required. To the other branch of the cannula was affixed an open rubber tube 3—4 cm in length. The cat breathed spontaneously throughout the experiment, but at each respiration inspired both a mixture of ether and air from the pump and fresh air from the free branch of the cannula. The depth of anesthesia was roughly adjusted by varying the ether content of the air from the pump. Fine adjustment was possible by varying the length of the rubber tube on the free branch of the Y-cannula. In the later experiments — though not the quantitative ones — the cats breathed via a small Krogh spirometer of 500 ml, filled with O_2, in which ether was dripped until the required depth of anesthesia had been obtained. This type of anesthesia was superior to the former,

partly because the depth was more constant and partly because the animal was well oxygenated, the basal lactic acid content of the blood thus being low. During preparation of the animal, it was anesthetized so deeply that only very slight cutaneous reflexes or none at all were elicitable. After the completion of preparation the anesthesia was relaxed to the stage where there were incipient spontaneous muscle movements, after which it was once more deepened to the point where they just disappeared; for spontaneous muscle movements in the extremities caused substantial variations in the blood flow.

The cat was thereafter prepared as follows. A T-cannula was introduced into the left carotid artery, and from it the blood pressure was recorded by mercury manometer and — after aspiration of 1 ml blood — samples were taken for determination of the lactic acid, oxygen and carbon dioxide contents of the arterial blood.

In the initial series of experiments the blood flow was measured from the femoral vein, into which a glass cannula had been inserted 2—3 cm proximal to the femoral opening in the adductor. In the subsequent experimental series, however, the blood flow was determined from the right brachial vein, into which a T-cannula had been introduced about 3 cm proximal to the elbow. From one branch of the cannula the blood was conducted to a drop recorder *ad modum* CLEMENTZ & RYBERG (1949) and thence to a graduated glass. The volume of outflowing blood was determined for 5-minute periods. Samples for lactic acid assay of the venous blood were taken from that blood. In the experiments in which the muscle respiration, too, was determined, blood was continuously aspirated from the other branch of the T-cannula. Aspiration was done with a small pump at the rate of 2 ml per 5 minutes. The blood was pumped into a paraffinized glass syringe containing a drop of mercury. When the syringe had been filled, it was sealed by placing a rubber cap over the tip. Prior to assay of the oxygen and CO_2 contents, effective mixture of plasma and the blood cell fraction was possible by shaking the syringe, the drop of mercury serving as a stirrer.

For intra-arterial infusion of adrenaline and the lactic acid solutions a fine cannula was inserted, in the first experiments, into the great saphenous artery. However, the adrenaline solution, containing 2 μg/ml, was found to produce marked local constriction of both the saphenous artery and adjacent parts of the femoral artery. That vasodilator effect of adrenaline which involved more peripheral areas was, therefore, either of very short duration or quite absent. In order to eliminate this complication it was necessary to insert a T-cannula, about 1 cm long, into the artery. The blood flowed through the horizontal branches and adrenaline was infused via the vertical branch. When the adrenaline solution was thus diluted by the blood, only very slight vasoconstriction or none at all occurred. The arterial cannula was introduced at the same level as the venous one.

Adrenaline and the lactic acid solutions were administered by means of an infusion apparatus. The solvent consisted of Macrodex (Pharmacia), which is a 6 per cent solution of dextran in physiological

saline with a pH of 7.0. Macrodex solution of room temperature, infused intra-arterially in a dose of 0.1 ml/min, produced only transient vasodilatation or none at all. This rate of infusion was consistently employed throughout the experiments. At higher infusion rates there was a slight increase in blood flow of short duration. In order to prevent unduly rapid oxidation of the adrenaline the macrodex solution was saturated with nitrogen. Adrenaline solution thus treated retained its full activity for at least two hours. The solution for infusion contained 2.5—4 μg/ml adrenaline and was prepared with synthetic l-adrenaline (Bios). The lactic acid, too, was dissolved in macrodex. It was prepared from dl-lactic acid which, after dilution with 10 volumes of distilled water, was boiled under reflux for 6 hours in order to break down the anhydrides (PURDIE & WALKER, 1892). The lactic acid content of this solution was assayed both by titration with 1N NaOH and by analysis *ad modum* FRIEDEMAN & GRAESER (1933). The blood leaving the femoral or brachial vein was returned by intravenous drip via a cannula inserted into the right jugular vein. To compensate the blood loss caused by the collection of blood in 5-minute periods and the removal of samples, the container for the intravenous drip was filled with 50 ml of macrodex solution. The latter was infused prior to the experiment. This dilution of the blood with macrodex was distinctly reflected in a decrease of the oxygen and CO_2 contents. By increasing or reducing the rate of the intravenous drip it was possible to keep the blood pressure variations within 10 mm Hg plus or minus. However, in those experiments in which the blood flow was determined from the femoral vein, the blood pressure could not be prevented from falling by up to 30 mm Hg during the infusion of adrenaline. This was partly because of a very pronounced rise in the blood flow — by up to 20 ml/min — and partly because some adrenaline probably reached the systemic circulation via collaterals and had a depressor effect.

In some of the initial experiments on the hind legs of cats the skin was separated from the muscle by blunt dissection, and communicating vessels were ligated. The skin was thereafter replaced round the muscle. This traumatization nevertheless sufficed to reduce or abolish the vasodilator effect of adrenaline. In the later experiments on the forelegs, therefore, only the venous anastomosis between the brachial and cephalic veins in the elbow fold was ligated. That branch from the brachial artery which supplies the skin and runs together with this venous anastomosis, was also ligated. In order to ascertain whether any further anastomoses existed between the deep and superficial venous systems of the foreleg, the orifice of the cannula conducting the blood from the vein to the drop recorder was raised. If the blood flow decreased, it implied that further anastomoses were present, in which case they were localized and ligated.

The lactic acid content of the skeletal muscle was determined as follows. After infusion of adrenaline or lactic acid both forelegs were amputated at the elbow, the motor nerves being cut first in order to prevent the lactic acid content from increasing in the muscle due to contraction of the latter. The skin was then removed and the extrem-

ities immersed in a mixture of ether and carbon dioxide ice. The interval between the completion of infusion and this cooling of the muscles amounted to about 1 minute. When the extremity was frozen through, it was removed from the freezing solution and cut into thin slices, which were then submerged in a tared beaker containing 30 ml of 10 per cent ice-cooled trichloracetic acid, whereupon the whole was weighed. Only the muscles of the lower foreleg were taken for analysis. They were then homogenized with a Bühler apparatus, after which a further 30 ml trichloracetic acid was added. The solution was centrifuged, and from the clear supernatant fluid were taken samples of 15 ml for lactic acid assay *ad modum* FRIEDEMAN & GRAESER (1933). Each muscle was subjected to three duplicate determinations. The lactic acid content of the blood was assayed by the same method, with duplicate determinations on 1.5 ml blood.

The O_2 and CO_2 contents of the blood were determined in 0.5 ml samples by the manometric method of VAN SLYKE (PETERS & VAN SLYKE, 1932).

Results

The vasodilator effect of adrenaline was studied in a total of 70 cats. The initial infusion of adrenaline in any one animal generally had the greatest and longest effect; with repeated infusions the effect progressively diminished unless they were given at sufficiently long intervals: 15—30 minutes. A vasodilator effect was demonstrable in most experiments, but it varied substantially in both magnitude and duration. In animals presenting the greatest effect, the blood flow increased up to threefold and this rise persisted for up to 20 minutes. In general, however, both the increase and its duration were less (see Table IV). Worthy of note was that the vasodilatation did not necessarily coincide with the infusion of adrenaline; in some cases the increase of blood flow subsided before (Fig. 1), and in others persisted for several minutes after, the infusion of adrenaline had terminated. If, however, the infusion was sufficiently prolonged, the vasodilator effect sooner or later ceased. In some cases a period of vasoconstriction followed the completion of infusion, when the increase in blood flow had subsided, before the initial level was again reached.

Of those factors which, in addition to the anesthetic, influenced the vasodilator effect of adrenaline, the most important were the duration and extent of preparation. If, due to technical difficulties, more than $1\,^1/_2$ hours elapsed from the onset of anesthesia to the start of the experiment, the effect of adrenaline

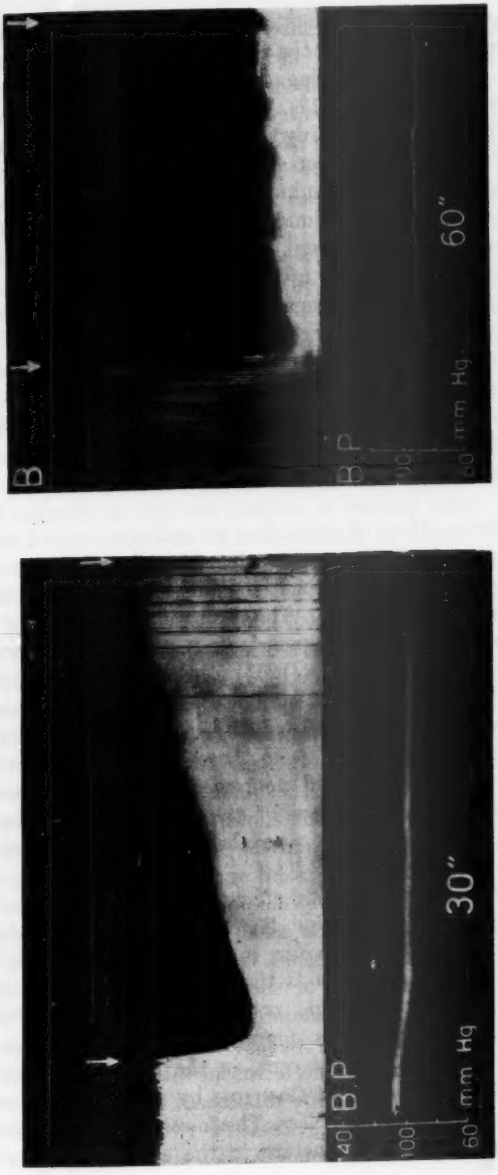

Fig. 1. Upper curve: Blood flow from the right brachial vein registered by an ordinate recorder. The amplitude increases with decreased rate of drip, but falls with increased blood flow. Lower curve: Blood pressure from the carotid artery.

A. Ether-anesthetized cat, 4.1 kg. Basal blood flow 3.2 ml/min. Between the arrows, infusion of 0.4 μg adrenaline per minute for 15 minutes into the brachial artery.

B. Cat, 3.8 kg. Basal blood flow 1.4 ml/min. Between the arrows, intra-arterial infusion of 0.27 mg dl-lactic acid per minute.

†2—563449

was either weak or quite absent. Traumatization or cooling of the foreleg muscles also caused the vasodilator effect of adrenaline to disappear, this finding being consistent with that reported by HARTMAN & FRASER (1917). In these cases the depressor action of adrenaline could nevertheless be produced by intravenous injection, and if another extremity was prepared a vasodilator effect could also be induced in several cases by intra-arterial infusion. In some animals where the vasodilator effect of adrenaline was not demonstrable immediately after preparation, it nevertheless appeared after the cat had lain for 30—60 minutes. Importance also attached to the initial vasotonia. If the blood flow was initially high, due *e. g.* to overheating of the animal, the vasodilator effect of adrenaline was either slight or absent. If the whole animal was cooled and the vascular tonus increased, the vasodilator effect was pronounced, as reported by REIN & SCHNEIDER (1930). It was substantial, too, when the vascular tonus was raised by subjecting the animal to acute anoxia.

The vasodilator effect of adrenaline accordingly seemed to be an unstable physiologic phenomenon, readily affected by various traumatizing factors, as was recently pointed out by GOTTSTEIN, HILLE & OBERDORF (1955) too. This probably explains why not all authors have been able to demonstrate it. In the investigations conducted by BARCROFT *et al.* (1953) adrenaline usually had a vasodilator effect in skeletal muscle in experiments on unanesthetized human subjects with a method causing very little traumatization of vessels and muscles. — In those cases where the vasodilator effect of adrenaline did not emerge in experiments on cats, the traumatizing influence of the method was probably responsible. In cases where it was absent, the vasoconstrictor action appeared instead.

Lactic Acid. The vasodilator effect of lactic acid was discovered by GASKELL (1880) and has since been verified by a number of workers. Only in a few investigations, however, has it been studied by such methods that the results may be considered valid under physiologic conditions. FLEISCH & SIBUL (1933), in experiments on cats, found that with an increase of 4—8 mM, *i. e.*, 32—72 mg per cent, in the lactic acid content of the arterial blood, the blood flow rose by up to 300 per cent, though in general the rise was less. The lowest active concentration was 3—4 mM. Sodium lactate in the same concentration

as lactic acid had only a very slight vasodilator effect. DOMINI & REIN (1942, 1943) found in dogs that on infusion into the femoral artery, a fivefold increase of the lactic acid content of the arterial blood — from 20 to 100 mg per cent — served to raise the blood flow by up to 300 per cent. In some cases, however, either no effect or vasoconstriction was obtained. Sodium lactate in the same concentration generally had a more powerful vasodilator effect than lactic acid.

On infusion of lactic acid into the femoral or brachial artery the blood flow increased in the majority of experiments. The lowest active dose in infusion into the brachial artery was 0.05—0.1 mg/min, corresponding to a rise of 2.5—10 mg per cent in the lactic acid content of the arterial blood. Maximal increase of the blood flow was obtained on infusion of 0.3—0.4 mg/min, corresponding to a rise of 10—30 mg per cent in the lactic acid content of the arterial blood when the basal blood flow amounted to 1.5—3 ml/min. When the lactic acid content was doubled to 0.8 mg/min, it did not produce a greater effect. The maximal increase of the blood flow amounted to about 300 per cent of the initial value. In general, however, the increase was smaller and, notwithstanding continued infusion, the blood flow sooner or later returned to the initial level. In those cases where adrenaline had no vasodilator effect, lactic acid increased the blood flow only very slightly or not at all, and in some of them it produced vasoconstriction. In all cases in which adrenaline exerted a vasodilator effect, even lactic acid served to increase the blood flow. A direct comparative investigation of the vasodilator effects of adrenaline and lactic acid on the same animals was difficult, since with repeated infusions the effect progressively subsided. Only if intervals of 15—30 minutes were allowed between infusions did the vessels have the same sensitivity. The first infusion of lactic acid or adrenaline, however, generally had the strongest effect. During the initial phase of the infusion, adrenaline nevertheless seemed to exert, in general, a somewhat greater dilator effect than lactic acid; while in the later phase of infusion it was vice versa. Various traumatic factors that inhibited the vasodilator effect of adrenaline did the same with lactic acid.

The lactic acid concentrations that, in this investigation, exerted a maximal vasodilator effect were five to ten times lower than those found by FLEISCH & SIBUL (1933) and DOMINI & REIN (1942/43). This disparity may well be attributable, at least

Table I.

The effect of adrenaline on blood flow and lactic acid production of the hind leg of the cat. Adrenaline was infused for 5 minutes in the right femoral artery.

Cat no. and weight kg	Adrenaline dose μg/min	Blood flow		Lactic acid production			
				Basal period		Infusion of adrenaline	
		Basal ml/5 min	Increase per cent after adrenaline	Arterio-venous difference mg per cent	Elimination mg/5 min	Arterio-venous difference mg per cent	Increase of elimination mg/5 min
no. 1 1.75 kg	0.45	14	140	+ 0.6	— 0.08	— 7.3	2.53
	0.45	15	113	— 9.3	1.40	— 7.7	1.06
	0.45	16	87	— 10.9	1.69	— 6.5	0.20
no. 2 3.6 kg	0.93	27	133	— 4.6	1.24	— 0.5	— 0.92
	0.93	44	82	— 4.3	1.89	— 0.7	— 1.33
no. 3 3.2 kg	0.93	33	8	— 6.6	2.15	— 0.7	— 1.90
	0.45	31	61	— 8.2	2.54	— 8.2	1.56
no. 4 2.0 kg	0.45	18	23	— 11.0	1.93	— 6.4	— 0.55
Mean			81	— 6.8	1.60	— 4.8	0.08

in part, to the fact that the operative measures in my experiments were reduced to a minimum — a precaution which the above-mentioned authors do not appear to have observed.

These qualitative experiments on the vasodilator effect of adrenaline and lactic acid accordingly showed that both the degree and the duration of vasodilatation were alike in intra-arterial infusion of the two substances. The doses of lactic acid that were required in order to produce a degree of dilatation equal to that induced by adrenaline were not greater than the amounts which adrenaline could be expected to liberate.

Quantitative Experiments. In order to establish if the lactic acid producing effect of adrenaline was responsible for its vasodilator action, it was necessary to determine the amount of lactic acid formed at infusion of adrenaline. In the first series of experiments I determined only the increase in that lactic acid which left the muscle with the venous blood during infusion of adrenaline. The results of the experiments, performed on one hind leg of cats, are detailed in Table I. The blood flow, it will be seen, increased on the average by 81 per cent of the initial value during the 5-minute period in which adrenaline was infused. The basal blood flow had been determined during the five minutes immediately preceding the infusion. The basal arterio-venous difference in lactic acid averaged — 6.8 mg per cent, and this *fell* during the infusion of adrenaline to — 4.8 mg per cent, because the lactic acid content of the venous blood decreased. The total lactic acid output from the muscle rose, therefore, only from 1.60 mg to 1.68 mg/5 min, *i. e.*, by 1 per cent. The results could be interpreted to imply either that adrenaline did not induce any lactic acid formation in the muscle, or that the method employed was unreliable. The latter was found to be the case. From the experiments in which adrenaline was infused intravenously (see Part II) it was estimated that adrenaline gave rise to the production of about 6 mg lactic acid per 100 g tissue per 5 minutes. The vascularized area distal to the infusion site in the femoral artery weighed about 100 g in cats having body weights of 3—3.5 kg. When lactic acid was infused into the femoral artery in a dose of 5—6 mg/5 min, the blood flow increased by an average of 54 per cent (Table II). In these experiments, too, there was a decrease of both the lactic acid content of the venous blood and the arterio-venous difference in lactic acid. The total lactic acid output rose by a mere 0.19 mg/5 min,

Table II.

The effect of lactic acid on blood flow and lactic acid production of the hind leg of the cat. Lactic acid was infused for 5 minutes in the right femoral artery.

Cat no. and weight kg	Lactic acid dose mg/min	Blood flow		Lactic acid production			
				Basal period		Infusion of lactic acid	
		Basal ml/5 min	Increase per cent after lactic acid	Arterio-venous difference mg per cent	Elimination mg/5 min	Arterio-venous difference mg per cent	Increase of elimination mg/5 min
no. 2 3.6 kg	1.1 1.1	34 36	59 37	— 4.5 — 2.5	1.53 0.89	1.6 — 5.3	— 2.39 1.68
no. 3 3.2 kg	1.2 1.2	48 50	111 39	— 6.4 — 6.1	1.44 2.20	— 5.0 — 7.4	0.94 1.50
no. 5 3.7 kg	1.2	46	25	— 7.4	3.37	— 4.5	— 0.80
Mean			54	— 5.4	1.89	— 4.1	0.19

i. e., only about 3 per cent of the amount administered was recoverable.

These experiments indicated that some formed or infused lactic acid was probably retained in the muscle, and that some of the blood from the femoral artery left the muscle via collaterals of the femoral vein. The role of the collateral circulation was evident from the fact that if the venous pressure was elevated by raising the orifice of the outlet cannula, the blood flow decreased substantially. With the rise in venous pressure that coincided with the increased blood flow after infusion of adrenaline or lactic acid, a greater portion of the blood was probably conducted through the collaterals.

In the search for a vascular area where the venous collateral circulation was negligible and, at the same time, the muscle could be readily assayed for lactic acid, experiments were conducted on the gastrocnemius muscle. However, the traumatization during preparation proved to be so great that adrenaline had only a vasoconstrictor action. Experiments were performed instead on the region supplied by the brachial artery. The blood coming from that artery should be possible to collect almost completely in the brachial vein after ligation of the anastomosis of the cutaneous venous system in the cubital fold; for the amount of lactic acid infused into the artery could be quantitatively recovered in the venous blood. Yet it is probable that a venous collateral circulation existed in this preparation too; for if the brachial artery was clamped, the blood flow from the vein decreased by about one half. With such a functionally efficient arterial collateral circulation, even the venous collaterals should be well developed. Under the relevant experimental conditions, however, it did not seem to be of any major importance.

The experiments on cats' forelegs were performed as follows. After preparation, an interval of 15—20 minutes was allowed for the blood flow to become constant. The basal blood flow was then measured for a 5-minute period, and at the same time blood was continuously collected for determination of the oxygen and CO_2 contents. From the blood in the graduated glass, samples were taken for lactic acid assay of the venous blood. In the middle of the 5-minute period, arterial blood was taken from the carotid for gas analysis and lactic acid assay. The infusion of adrenaline, lactic acid or, in the control experiments, macrodex solution commenced immediately thereafter. The blood flow was

determined for two or, in some of the adrenaline experiments, for three 5-minute periods, during each of which samples were taken for gas and lactic acid assay. At the start of the second 5-minute period, samples were also taken of the arterial blood. Immediately after the infusion both the right foreleg — in which the infusion had been given — and the left one were amputated at the elbow, and the muscle of the lower part was analyzed for lactic acid.

Basal Values. The values for blood flow, lactic acid production, oxygen consumption and CO_2 production are given per kilogram of muscle in the lower foreleg. The contribution from the skeleton to the above-mentioned physiologic magnitudes was considered negligible. The basal blood flow in 24 experiments on different cats averaged 107.7 ml per kg muscle per minute, the range being 48.6—193.1 ml. The observed blood flow was twice to thrice that reported by BARCROFT & SWAN (1953) in man. Since the cat's oxygen consumption per kilogram of body weight is two to three times greater than that in man, differences in blood flow of the same order of magnitude seem probable. — The average oxygen consumption was 5.6 ml/kg muscle/minute. The total oxygen consumption of the ether-anesthetized cat was calculated at 8 ml per kilogram of body weight (see Part II). Hence the oxygen consumption in the muscle was of the same magnitude as the total oxygen consumption. The mean CO_2 production amounted to 4.8 ml/kg muscle/minute. The respiratory quotient was 0.98. The lactic acid content of the arterial blood averaged 21.9 mg per cent, the range being 7.8—51.6. In Part II it will be shown that lactic acid values exceeding 20 mg per cent are not strictly basal; the basal content was undoubtedly somewhat elevated in these experiments, due, no doubt, to the depth of anesthesia. In order to prevent spontaneous muscle movements attended by variations in the blood flow, the animals had to be kept under relatively deep anesthesia. In the experiments reported in Part II, where the animals were not anesthetized so deeply, the basal lactic acid contents were consistently lower. The mean lactic acid content of the venous blood was 29.0 mg per cent; *i. e.*, the arterio-venous difference in lactic acid averaged — 7.1 mg per cent, the range being 1.4—17.4 mg per cent. — HIMWICH, KOSKOFF & NAHUM (1930), in experiments on amytal-anesthetized or decerebrated dogs, found that the arterio-venous difference in lactic acid in limb blood

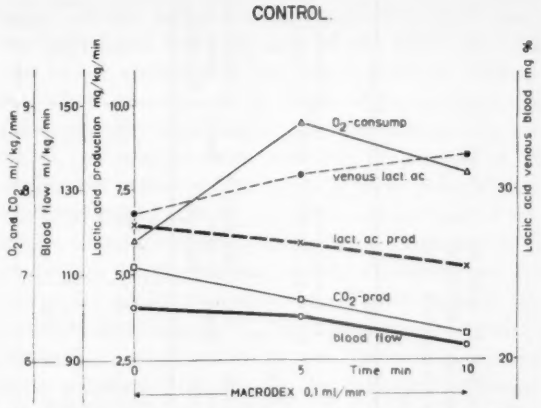

Fig. 2. Effect of 0.1 ml macrodex per minute, in intra-arterial infusion, on the blood flow, lactic acid production, O_2 consumption and CO_2 production in the foreleg muscles and on the lactic acid content of the venous blood. Each point is the mean of four to six determinations.

exceeded 5 mg per cent in 24 of 38 experiments. In unanesthetized dogs or human subjects the arterio-venous difference in no case exceeded 5 mg per cent. CHERRY & CRANDALL (1939), in 16 similar experiments on unanesthetized dogs, found a mean difference of — 3.1 mg per cent. — Even with due regard to the fact that in my experiments the venous blood came from the muscles, whereas in those of HIMWICH, KOSKOFF & NAHUM and CHERRY & CRANDALL it was mixed with blood from the skin, the disparities in the arterio-venous difference in lactic acid are so great that the ether anesthesia must be assumed to have had some stimulating effect on the lactic acid production. This view is consistent with the aforegoing considerations as to the lactic acid content of arterial blood. — The mean lactic acid production amounted to 5.9 mg/kg muscle/minute. From this value and the oxygen consumption it can be calculated that the total energy metabolism of the muscles was $3.0 \cdot 10^{-3}$ Cal/kg muscle/minute, and that 8 per cent thereof was referable to glycolytic processes.

Control Experiments. In six experiments macrodex alone was infused at the rate of 0.1 ml/min for 10 minutes. In these experiments (Table III, Fig. 2) the blood flow fell somewhat, on the average, during infusion. The lactic acid and the CO_2 production also declined in some degree, but the oxygen consumption showed

Table III.

Control tests. Macrodex (0.1 ml per min) was infused for 10 min in the brachial artery. Effect on lactic acid content in the muscle, total lactic acid production, oxygen consumption, carbon dioxide production and blood flow of the anterior limb of the cat.

Cat weight kg	Blood flow ml/kg muscle/min			Lactic acid content in muscle mg per cent		Lactic acid production mg/kg muscle/min			O_2-consumption ml/kg muscle/min		CO_2-production ml/kg muscle/min	
	Basal	Infusion of macrodex				Elimination with the blood		Total deviation in production after Macrodex				
		0—5 min	5—10 min	Control	Deviation after Macrodex	Basal	Deviation after Macrodex		Basal	Deviation after Macrodex	Basal	Deviation after Macrodex
2.4	88	100	100	25.1	— 3.0	3.1	— 2.0	— 4.9	—	—	—	—
2.5	97	110	83	38.1	0.6	17.0	0	0.6	3.4	3.5	4.6	0
3.3	190	158	168	34.9	3.1	6.1	— 0.2	1.3	12.7	— 1.4	10.0	— 4.4
3.6	53	44	35	35.7	1.7	2.9	1.7	4.1	—	—	—	—
3.6	139	122	135	44.2	6.2	4.4	2.0	7.6	2.5	1.1	1.8	2.0
3.0	49	65	38	77.3	— 18.7	5.4	2.0	— 13.9	3.0	1.0	3.9	— 0.2

no change. In this and following figures the lactic acid production was calculated from the sum of the lactic acid discharged into the blood stream and the changes of its content in the muscles, where the amount in those of the control extremities was taken as the basal value. The changes in the lactic acid content of the muscles were assumed to proceed at the same rate during the infusion. The content in muscles of the control extremities averaged 42.6 mg per cent, and in those of the infused extremities 40.9 mg per cent. The total mean change in the lactic acid production during infusion, and its standard error, amounted to -0.9 ± 3.1 mg/kg muscle/minute. The changes in blood flow, lactic acid production and CO_2 production were so small that they were probably due to random factors. Infusion of macrodex thus caused no significant changes in the various physiologic magnitudes that were determined; nor were any changes in the lactic acid content of the muscles likely to have resulted from preparation of the extremities in which the infusions were given.

Adrenaline Experiments. This series comprised 12 experiments with one infusion of adrenaline in a dose of 0.3—0.4 μg/min, the equivalent of 11.3—19.0 μg/kg muscle/min. This dose of adrenaline had a maximal vasodilator effect on *ether-anesthetized* cats. It should be pointed out that with the use of other anesthetics, such as chloralose, the same dose would have a pronounced vasoconstrictor action (see Part II). In two of these experiments the vasodilator effect of adrenaline was of such short duration that no analyses were carried out. In a further two experiments only the vasoconstrictor effect appeared, and although analyses were done in these cases they were not included in the calculation of the mean values. The remaining eight experiments, in which adrenaline produced a distinct increase of blood flow lasting at least five minutes, are detailed in Table IV, the mean values being shown in Fig. 3. In four experiments adrenaline was infused for 15 minutes; in the remaining four, for 10 minutes. From Fig. 3 it will be seen that the blood flow increased from a basal value of 108.3 ml/kg muscle/min to 168.4 ml during the first five minutes of the infusion. The increase persisted for a further five minutes, but 10—15 minutes after the start of infusion the blood flow had returned to the initial level.

The lactic acid content of the venous blood was not changed during the increase in blood flow. Not until the blood flow had returned to the initial level did the lactic acid content of the

Table IV.

Adrenaline tests. Adrenaline (0.25—0.4 μg per min) was infused for 10—15 min in the brachial artery.

Cat weight kg	Adrenaline μg/kg muscle/min	Blood flow mg/kg muscle/min				Lactic acid content in muscle mg per cent		Lactic acid production mg/kg muscle/min			O_2consumption ml/kg muscle/min		CO_2-production ml/kg muscle/min	
		Basal	Infusion of adr					Elimination with the blood		Total increase of production after adr				
			0—5 min	5—10 min	10—15 min	Control	Increase after adr	Basal	Increase after adr		Basal	Deviation after adr	Basal	Deviation after adr
4.1	19.0	151	252	195	146	45.1	38.2	9.6	— 1.5	17.8	10.8	— 3.8	11.5	— 5.2
4.5	14.8	94	156	200	111	40.8	0.1	8.7	5.8	6.2	4.6	1.4	7.7	4.2
4.0	17.0	94	102	111	94	40.2	4.5	3.0	3.0	6.0	3.5	— 0.5	4.0	1.8
3.7	15.4	51	67	77	56	47.5	9.4	— 0.7	4.6	10.8	4.3	— 0.7	3.6	0.8
4.3	15.4	133	181	92	—	27.1	6.2	0.7	1.4	7.6	11.6	— 2.5	8.0	— 1.4
3.8	11.3	113	143	151	—	40.3	4.8	4.0	5.0	9.8	5.4	1.1	4.5	2.1
2.8	12.8	78	210	200	—	47.8	2.6	9.4	10.5	13.2	2.4	0.4	3.8	5.0
2.4	15.2	152	236	212	—	58.5	0.4	11.9	3.2	3.6	7.9	— 3.6	4.6	6.5
[1] 4.7	12.1	173	161	91	—	27.2	16.7	1.8	0.8	17.5	9.2	— 1.2	8.2	— 1.1
[1] 3.0	13.2	153	147	100	—	48.5	6.5	4.8	3.6	10.2	11.1	— 3.4	10.8	— 1.4

[1] tests with diminished blood flow.

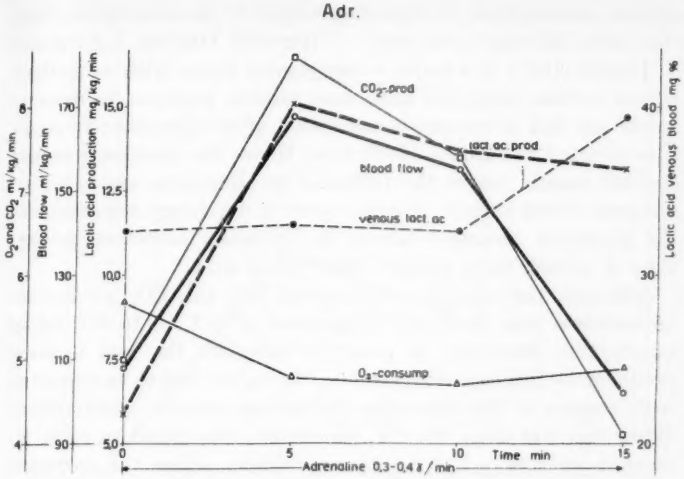

Fig. 3. Effect of adrenaline, in intra-arterial infusion, on the blood flow, lactic acid production, O_2 consumption and CO_2 production in the foreleg muscles and on the lactic acid content of the venous blood. Between zero and 10 minutes, each point is the mean of eight determinations; between 10 and 15 minutes, the mean of four determinations.

venous blood rise. This result was consistent with the findings of BELL & STEAD (1952) in experiments on human subjects. When the blood flow increased, however, the elimination of lactic acid from the muscles was almost doubled, although the arterio-venous difference in lactic acid showed no change. The increased elimination of lactic acid still remained after the blood flow had returned to the initial level. The lactic acid content of the muscles also rose, from 43.4 to 51.7 mg per cent, during infusion of adrenaline. The total lactic acid production increased from 5.8 mg/kg muscle/min during the basal period to 15.2 mg/kg muscle/min during the infusion; *i.e.*, by 9.4 ± 1.62 mg/kg/min (0.10 mM/kg muscle). The increase was statistically significant.

The oxygen consumption fell from a mean of 6.3 ml/kg muscle/min in the basal period to 5.3 ml during the infusion of adrenaline, but the fall of 1.04 ± 0.73 was not statistically verifiable. The increase in total energy metabolism of the muscle that was induced by the elevated lactic acid production amounted to $3.7 \cdot 10^{-3}$ Cal/kg muscle/min, and it was fairly consistent with the decrease in energy metabolism of $5.7 \cdot 10^{-3}$ Cal which attended the fall of

oxygen consumption, thus lending weight to the assumption that this latter fall was a real one. — GRIFFITH, OMACHI, LOCKWOOD & LOOMIS (1947), in a major experimental series with a method similar to that employed here, were unable, however, to demonstrate any fall in oxygen consumption after adrenaline in doses that elevated the muscle blood flow. Hence the question whether skeletal muscle, under the influence of adrenaline and with an adequate blood supply, covers a part of its energy requirements via glycolytic processes cannot be definitely answered as yet, even if certain facts suggest that this is so.

Although the oxygen consumption fell, the CO_2 production nevertheless rose from an initial level of 5.9 ml to 6.6 ml/kg muscle/min. However, in order to calculate the real increase produced by infusion of adrenaline, the values had to be corrected with respect to the coincident fall in the oxygen consumption. When this was done the CO_2 production was found to have increased by 2.56 ± 1.10 ml/kg muscle/min, where the corrected value for the CO_2 production was computed for each 5-minute period in the adrenaline experiments. The increase was statistically probable, which finding is consistent with the results obtained by CAMMER & GRIFFITH (1939) and GRIFFITH, OMACHI, LOCKWOOD & LOOMIS (1947). The rises in the CO_2 production and the blood flow were virtually parallel; when the increase in blood flow had disappeared, the CO_2 production had also returned to the initial level. The case was different with the lactic acid production, which was still elevated when the increase of blood flow had subsided.

The analytical results in the two experiments in which adrenaline reduced the blood flow are shown in Table IV. In both experiments a substantial increase of the lactic acid production was induced, so that the absence of vasodilatation could not be attributed to suppression of that production. The basal blood flow, on the contrary, was strikingly high in both experiments, and hence the vasoconstrictor effect may be assumed to have exceeded the vasodilator effect of adrenaline on these relatively atonic vessels.

Lactic Acid Experiments. On infusion of adrenaline the lactic acid production rose by an average of 9.4 mg/kg muscle/min. This latter was accordingly the amount that should have been infused into the brachial artery in order to find out whether the lactic acid produced in the adrenaline experiments was sufficient

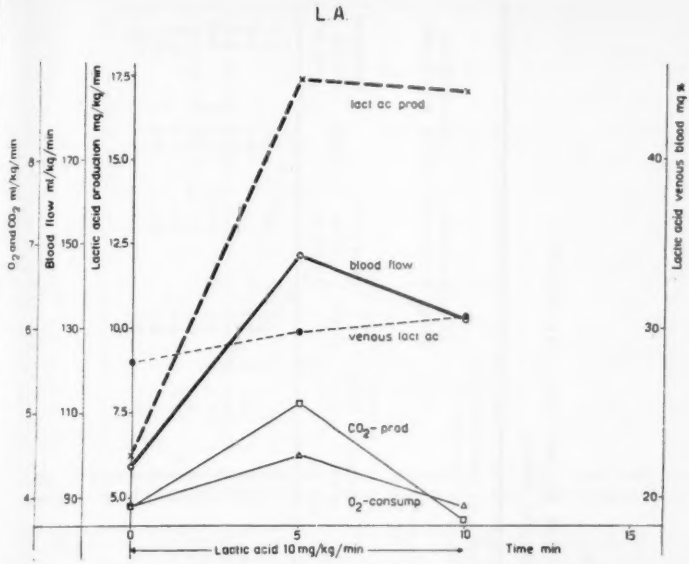

Fig. 4. Effect of intra-arterial infusion of lactic acid, 11.4 mg/kg muscle/min, on the blood flow, lactic acid production, O_2 consumption and CO_2 production in the foreleg and on the lactic acid content of the venous blood.

to induce vasodilatation. To make possible, in the relevant case, calculation of the amount of lactic acid to be infused, the ratio of the body weight to that of the right lower leg muscles had to be determined. In determinations on 15 cats this ratio averaged 6.2 g muscle to 1 kg body weight, the range being 5.1—7.4 g. In order, by administering lactic acid, to secure an apparently increased production as great as that in the adrenaline experiments, 0.058 mg lactic acid therefore had to be infused per kilogram of body weight per minute. But since the blood flow in the brachial artery was assumed to be probably somewhat greater than that in the brachial vein, a slightly larger amount was infused; namely, 0.07 mg lactic acid per kilogram of body weight per minute. The weight of the lower leg muscles and the body weight showed a somewhat lower ratio (6.12 g : 1 kg) in the lactic acid experiments. If the blood flow was identical in the brachial artery and vein, then this amount of lactic acid should have apparently increased the lactic acid production by 11.4 mg/kg muscle/min.

Table V.

Lactic acid tests. Dl-lactic acid (0.17—0.34 mg per min) was infused for 10 min in the brachial artery.

Cat weight kg	Lactic acid mg/kg muscle/min	Blood flow ml/kg muscle/min			Lactic acid content in muscle mg per cent		Lactic acid production mg/kg muscle/min			O_2-consumption ml/kg muscle/min		CO_2-production ml/kg muscle/min	
		Basal	Infusion of lactic acid				Elimination with the blood		Total increase in lactic acid				
			0—5 min	5—10 min	Control	Increase after lact ac	Basal	Increase after lact ac		Basal	Deviation after lact ac	Basal	Deviation after lact ac
2.3	12.4	55	85	77	49.6	13.3	8.8	4.4	17.7	3.1	2.0	2.3	0.8
4.8	12.9	123	158	177	22.4	3.3	4.8	2.1	5.3	3.2	1.1	3.3	0.8
4.7	11.3	193	248	238	29.1	6.9	3.9	8.8	15.7	7.7	— 1.9	8.3	— 1.3
3.4	11.6	49	78	59	33.3	13.3	3.0	5.4	18.7	2.0	— 0.1	1.7	0.9
2.7	11.8	100	125	106	46.7	— 3.4	5.1	3.2	0	3.8	2.3	6.3	— 2.6
3.8	11.1	59	192	158	40.7	6.0	4.5	11.2	17.2	3.6	0.4	3.8	0.7
2.9	11.6	120	189	154	56.0	— 2.0	12.5	7.5	5.5	3.7	0.3	3.8	2.1
4.0	9.5	82	105	85	36.9	5.7	7.0	2.6	8.3	3.7	— 1.3	1.7	2.6

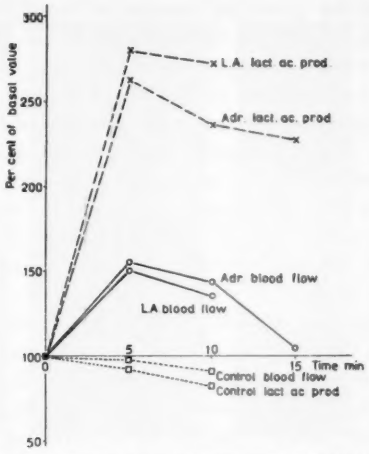

Fig. 5. Comparison of the mean percental increases of blood flow and lactic acid production in the adrenaline (Adr.), lactic acid (L. A.) and control experiments.

Eight experiments were conducted, in which lactic acid was infused over a 10-minute period. The results are shown in Table V and Fig. 4. In each case the blood flow rose. On the average it increased from a basal level of 98 ml to 147 ml/kg muscle/min during the first five minutes of the infusion, and in the second 5-minute period it amounted to 132 ml. The lactic acid content of the venous blood rose by only a few milligrams per cent, although lactic acid was infused at such a rate that the content of the arterial blood was calculated to have increased by an average of 10 mg per cent. The lactic acid content of the skeletal muscle showed a mean rise of 5.4 mg per cent. The lactic acid production showed a total apparent increase of 11.1 ± 2.52 mg/kg muscle/min. With an equivalent blood flow in the brachial artery and vein, the increase should have been 11.4 mg/kg muscle/min. The result indicated that the blood flow was equal in the artery and vein, and that the method employed quantitatively embraced the lactic acid production in the muscles.

The oxygen consumption showed no significant change. Its total increase amounted to 0.4 ± 0.52 ml/kg muscle/min. The CO_2 production, as in the adrenaline experiments, showed a mean rise of 0.5 ± 0.60 ml/kg muscle/min. The increase was, accordingly, less than that in the adrenaline experiments and was not statistically verifiable.

Fig. 5 shows the percental changes of blood flow and lactic acid production, in relation to the initial levels, in the different experimental series. The blood flow and lactic acid production showed very similar courses in the adrenaline and the lactic acid experiments, though in the latter the increase of blood flow was somewhat less. This last-named result was probably due to some measure of selection in the adrenaline series, for analyses were carried out only in those experiments where the increase of blood flow was pronounced and of long duration. Taking all experiments together, the rise in blood flow was somewhat greater in the lactic acid series.

Effect of Glycolysis-inhibiting Substances. MOHME-LUNDHOLM (1953) showed that various glycolysis inhibitors such as Cu^{++} ions, sodium fluoride, monoiodoacetic acid, dl-glyceraldehyde, sodium azide and sodium arsenate, blocked the relaxing effect of adrenaline on smooth muscle by suppressing its ability to produce lactic acid. It was worth while ascertaining whether any of these substances also blocked the vasodilator effect of adrenaline.

Copper ions were administered as $CuCl_2 \cdot H_2O$ in macrodex solution by infusion into the brachial artery at a rate of 0.2 mg/min. With unduly rapid infusion the blood flow fell very substantially, probably, in part, because the copper ions precipitated the blood protein, which then formed small emboli and occluded the minor vessels. When about 2 mg had been infused the blood flow had decreased to roughly one half, due to the vasoconstrictor effect of the copper ions. A dose of adrenaline which had previously caused vasodilatation now produced constriction (Fig. 6 B). It will be shown, in Part II, that this inverse adrenaline reversal was associated with blocking of the lactic acid producing effect of adrenaline.

Sodium fluoride was infused intra-arterially at the rate of 1—2 mg/min. The blood flow increased during infusion but thereafter fell by about 20 per cent. After 20 mg of sodium fluoride, a dose of adrenaline which had previously had a vasodilator action now produced vasoconstriction (Fig. 6 D). Lactic acid in an amount normally formed during infusion of adrenaline had, on the other hand, a vasodilator effect. About one hour after the infusion of sodium fluoride the dilator effect of adrenaline returned. — TAKEHIRO (1932) found that fluoride inhibited the stimulating effect of adrenaline on the lactic acid production in striated muscle.

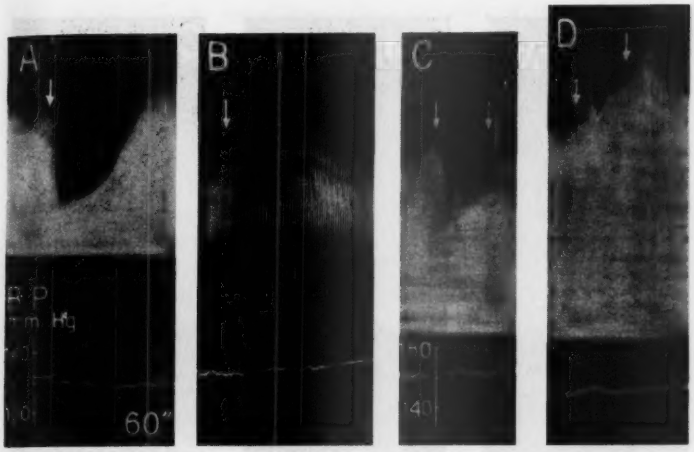

Fig. 6. A. Ether-anesthetized cat, 3.2 kg. Basal blood flow 1.8 ml/min. At the arrow, injection of 0.5 μg adrenaline into the brachial artery. B. Same animal after infusion of a total of 2 mg $CuCl_2 \cdot 2\ H_2O$ for 10 minutes into the brachial artery. At the arrow, 0.5 μg adrenaline intra-arterially. Basal blood flow 0.8 ml/min. C. Cat, 3.1 kg. Basal blood flow 1.7 ml/min. Between the arrows, intra-arterial infusion of 0.25 μg adrenaline/min. D. Same animal 20 minutes after infusion of 20 mg sodium fluoride with a rate of 2 mg/min. Between the arrows, 0.25 μg adrenaline/min. Basal blood flow 1.5 ml/min.

Whether monoiodoacetic acid, dl-glyceraldehyde and sodium azide were capable of blocking the vasodilator effect could not be decided with assurance; for all substances induced marked vasodilatation. In doses that increased the basal blood flow only very slightly, no definite blocking was demonstrable. Doses which themselves increased the blood flow blocked the dilator effect of adrenaline, but whether this was due to altered tonus of the vessels or to the glycolysis inhibiting effect of those substances could not be established.

Vasodilator Effect of the Lactate Ion. Since there was considerable evidence that the vasodilator effect of adrenaline resulted from its ability to form lactic acid in skeletal muscle, it seemed worth while finding out whether the vasodilatation was merely due to the decreased pH or whether the lactate ion too had some dilator effect. — In the opinion of KURTZ & LEAKE (1927) and DOMINI & REIN (1942), the lactate ion has a vasodilator effect, though MÜLLER (1924) and FLEISCH & SIBUL (1933) thought otherwise.

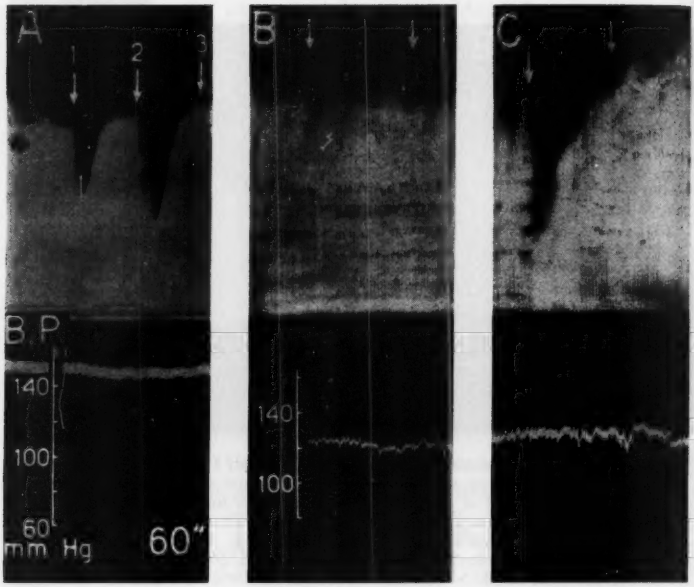

Fig. 7. A. Cat, 3.7 kg. Between 1 and 2, infusion of $2.6 \cdot 10^{-3}$ mM HCl/min (0.0007 ml 1 N HCl/kg body weight/min) into the brachial artery. Between 2 and 3, infusion of the equivalent amount of lactic acid (0.23 mg/min). Basal blood flow 2.0 ml/min.
B. Cat, 2.7 kg. Basal blood flow 1.8 ml/min. Between the arrows, intra-arterial infusion of 0.2 mg lactate/min in the form of sodium salt.
C. Same animal. Adrenaline has been infused for 10 minutes in a dose of 0.2 μg/min. When the blood flow had returned to the initial level concurrent infusion — between the arrows — of 0.2 mg lactate per min.

The experiments described below were performed on a total of 20 cats under ether anesthesia. The infusions were given in the right brachial artery, the blood flow being determined in the right foreleg. To establish whether the lactate ion had any specific dilator effect, equivalent amounts of hydrochloric acid and lactic acid were infused into the brachial artery. The smallest effective amount of both HCl and lactic acid was 0.03—0.04 mM/kg muscle/min. Lactic acid in the amount formed during infusion of adrenaline — 0.10 mM/kg muscle/min — appeared to have a somewhat greater dilator effect than the equivalent amount of HCl, but direct comparison was difficult on the same preparation, since with repeated infusions the effect progressively diminished. It will be seen from Fig. 7 A that despite continuous

infusion of HCl the vasodilator effect was only of short duration. If infusion of lactic acid was rapidly substituted for HCl when the latter's vasodilator effect had subsided, further dilatation occurred (Fig. 7 A). If, on the other hand, an equivalent amount of H_2SO_4 was substituted for HCl, no such dilatation appeared. Since the amount of hydrogen ions administered was virtually the same throughout the infusion (differences in the dissociation constants of HCl and lactic acid are of no importance at the blood pH), the return of dilatation had to be attributed to the lactate ion. The effect was most distinct with the amounts of HCl and lactic acid indicated in the legend.

On infusion of sodium lactate into the brachial artery in the amounts formed at infusion of adrenaline (0.1—0.2 mg/min), the effect varied somewhat. In most experiments the vasodilator action was weak or absent, but in experiments on three animals it was almost identical with that produced by an equivalent amount of lactic acid. These animals were under deep anesthesia, so that the lactic acid production in the muscles should have been high. Control infusions with equivalent doses of sodium bicarbonate had no effect; hence the vasodilatation had to be attributed to the lactate ion.

Where sodium lactate under basal conditions had only a weak dilator action or none at all, the effect could be considerably amplified if the lactate was administered during the course of intra-arterial infusion of adrenaline. The effect was especially conspicuous when the lactate was infused after the vasodilator action of adrenaline had subsided (Fig. 7 C). It will be seen from the latter figure, however, that the dilator effect of the lactate ion disappeared in spite of continuous infusion, which finding was assumed to imply that it occurred only with a change in the lactate content of the blood. In order to investigate the effect, on the blood flow, of a continuously increasing lactate ion content of the blood, the infusion had to be given intravenously.

On intravenous infusion of lactic acid in doses equivalent to the amounts estimated to be formed after intravenous infusion of adrenaline (5—10 mg/kg body weight; see Part II) vasodilatation occurred in the muscles only during the initial stage of infusion. It was then followed by vasoconstriction due to the stimulating effect of lactic acid on the vasomotor center. With coincident intra-arterial infusion of adrenaline the peripheral

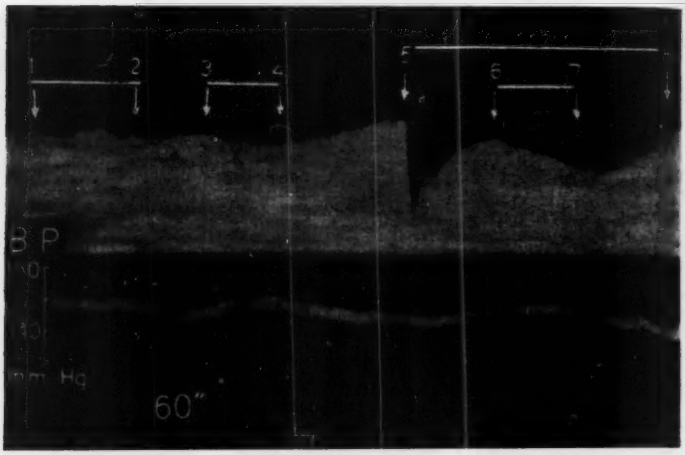

Fig. 8. Cat, 4.0 kg. Foreleg denervated two hours earlier. Between the arrows 1 and 2, intravenous infusion of 0.5 ml 0.6 mol NaCl. Between 3 and 4, intravenous infusion of 0.7 ml 0.6 mol sodium lactate solution (10 mg lactate/kg body weight/min). At 5 start of an intra-arterial infusion of 0.05 μg adrenaline/min. Between 6 and 7 concurrent intravenous infusion of 10 mg lactate/kg body weight/min. At 8 the end of the adrenaline infusion.

vasodilator effect preponderated, however, in some experiments, so that the vasodilator action of adrenaline was amplified. In an attempt to eliminate the central vasoconstrictor action of lactic acid, sodium lactate was instead infused intravenously. Under basal conditions it produced, in a dose of 10 mg/kg body weight (calculated as lactic acid), an increase of about 20 per cent in the blood flow, which rise was amplified by coincident intra-arterial administration of adrenaline. The lactate ion, however, probably had some central stimulating effect on the vasomotor center; for in cases where intra-arterial infusion of lactate had a pronounced dilator effect it was not possible to induce corresponding dilatation by intravenous injection or infusion of lactate, even though the lactate content of the blood may be expected to have increased to an equal degree in both cases. — It will be shown in Part III that anesthesia probably has a major influence on both the central and peripheral vascular response to lactic acid, and that in unanesthetized animals the peripheral vasodilator effect is apparently predominant.

For the purpose of completely eliminating the central vaso-

constrictor effect of lactate ions in anesthetized animals, experiments were conducted on acutely denervated extremities. About 60 minutes after denervation, when the blood flow had been stabilized, it was possible by intravenous infusion of sodium lactate to produced an increase in the blood flow, which rise was appreciably amplified by concurrent intra-arterial infusion of adrenaline (Fig. 8). This increase in blood flow on coincident infusion of adrenaline and lactate generally persisted as long as the lactate was infused, and only gradually returned to the initial level after completion of the lactate infusion. Generally it was necessary to carry out control infusions with equivalent sodium chloride solutions, for in some cases the hypertonic solution (0.3—0.6 N) that had to be infused produced some vasodilator effect. In a few experiments, moreover, the blood pressure rose by 10—20 mm Hg during the lactate infusion. The increase in blood flow nevertheless appeared regardless of whether the perfusion pressure was changed. In the experiments on denervated extremities it was necessary to administer much smaller amounts of adrenaline (0.02—0.05 μg/min) than those in intact extremities, for otherwise the vasoconstrictor effect appeared after a few minutes infusion.

The lactate ion accordingly had a dilator effect on muscle blood vessels that was particularly distinct after intra-arterial infusion. In cases where the lactic acid production in the muscle was increased, as in coincident infusion of adrenaline or under deep anesthesia, the dilator effect of the lactate ion was amplified.

Experiments on Isolated Vessels. The amounts of lactic acid produced on infusion of adrenaline were so great that they must have been formed in the skeletal muscle cells. On the other hand, it was conceivable that adrenaline also induced lactic acid production in the smooth muscle of the blood vessels and that this production, rather than that in the skeletal muscle cells, induced the vasodilator effect; for MOHME-LUNDHOLM (1956) had found that the tonus-reducing effect of adrenaline on isolated bovine coronary vessels was caused by lactic acid production in the smooth muscle. Hence it seemed worth while investigating, in cats, what influence adrenaline had on vascular areas not in contact with skeletal muscle. For this purpose a 4 cm long segment of the brachial artery was dissected free, and round the vessel, still *in situ*, cotton was applied and moistened, alternately, with Tyrode's solution of body temperature with and without adrenal-

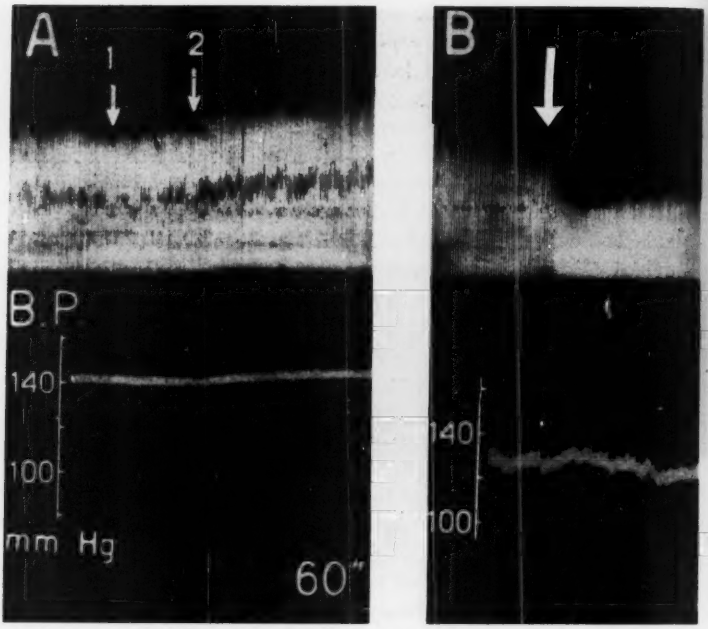

Fig. 9. A. Ether-anesthetized cat, 3.7 kg. Basal blood flow 1 ml/min. Four centimeters of the brachial artery had been dissected free. 1. Local application of Tyrode's solution at body temperature containing 0.1 μg adrenaline/ml. 2. Tyrode's solution containing 0.3 μg adrenaline/ml.
B. Ether-anesthetized cat, 2.5 kg. Local application to the brachial artery of carbaminoylcholine in Tyrode's solution (10 μg/ml). Basal blood flow 0.6 ml/min.

ine. The blood flow was determined in the usual way via a cannula inserted in the brachial vein. The concentration of adrenaline in the blood flowing through the brachial artery was estimated at 0.1—0.3 μg/ml during the infusion. When the cotton round the vessel was moistened with an adrenaline solution containing 0.1 μg/ml no effect was obtained, but after 0.3 μg/ml there was a gradual diminution of the blood flow (Fig. 9 A). That dilatation of the vessel was also reflected in the blood flow will be seen from Fig. 9 B, where local application of carbaminoylcholine or papaverine increased the flow. Adrenaline, as was consistent with the findings of earlier workers, thus had only a constrictor effect on skeletal muscle vessels when administered locally.

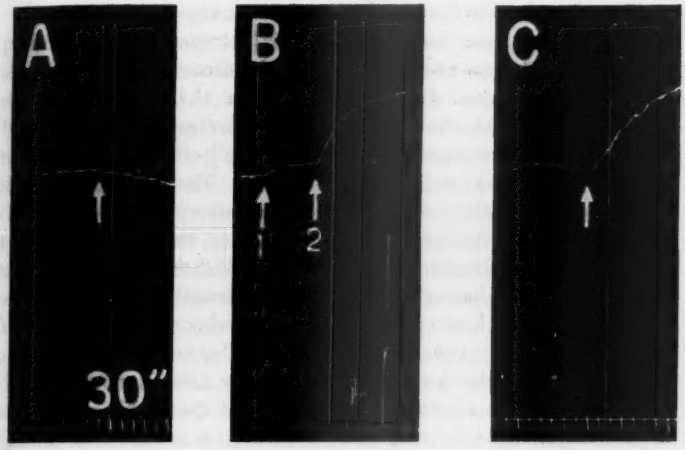

Fig. 10. Bovine renal artery in Tyrode's solution aerated with carbogen. Registration with an isotonic pen. A. Changes recorded in the direction of the circular muscle layer. At the arrow, adrenaline $2.5 \cdot 10^{-7}$ (5 μg/20 ml). B. Same preparation after removal of the tunica intima. 1. Adrenaline $2.5 \cdot 10^{-7}$. 2. Adrenaline $5 \cdot 10^{-6}$ (100 μg/20 ml). C. Same artery with the changes recorded in the direction of the longitudinal muscle layer. At the arrow, adrenaline $2.5 \cdot 10^{-7}$.

ROTHLIN (1920) found, however, that isolated renal arteries from cow were relaxed by adrenaline in low concentrations but constricted in high concentrations. FURCHGOTT (1955) called attention to a source of error in experiments on isolated vessels. Several workers (SCHMITT, 1922; HÄUSLER, 1933; SOLLMAN & GILBERG, 1938) observed that vessels having circular muscle fibers alone, lengthened at right angles thereto when treated with adrenaline or other vasoconstrictors. This phenomenon presented as "relaxation" if "tonus" was registered in the longitudinal direction of the vessels. Since BARBOUR (1912) had found longitudinal muscle fibers in bovine renal arteries, it seemed not unlikely that the "relaxation" of those arteries after small doses of adrenaline was due to contraction of the relevant muscle fibers.

In order to find out if this explained the opposite effect of adrenaline on isolated vessels, experiments were conducted on isolated bovine renal arteries. The arteries were grossly observed to possess an inner circular muscle layer and an outer, more powerful longitudinal layer. When longitudinal changes in the circular muscle layer of a square fragment of vessel wall were registered in an organ bath, adrenaline in a low concentration

was found to induce "relaxation", which changed to contraction when the adrenaline concentration was increased (Fig. 10). If, however, the change was registered in the direction of the longitudinal muscle layer, the adrenaline dose that had produced "relaxation" in the direction of the circular muscle fibers now induced marked contraction. The "relaxing" effect disappeared, even if the tunica intima was removed. The explanation of adrenaline's "relaxing" effect onthe renal artery may, therefore, be that the longitudinal muscle layer was more sensitive to adrenaline than the circular layer, and that on contraction of the longitudinal fibers the circular fibers lengthened.

Adrenaline has been reported both to relax and to contract pulmonary arteries too (WISSLER, 1931; CHANG, 1932), though independently of the dose. Since pulmonary arteries also possess longitudinal fibers (BENNINGHOFF, 1950), it seems likely that a similar explanation is valid for the varying reaction of those vessels to adrenaline.

Discussion

Localization of the Receptors for the Vasodilator and Vasoconstrictor Effects of Adrenaline

According to the theory originally propounded by DALE (1906) and subsequently elaborated and modified by AHLQUIST (1948), LANDS (1952) and FURCHGOTT (1955), among others, smooth muscle contains two types of receptors; one of them already combined with adrenaline in low concentrations, leading to relaxation of the muscle (inhibitor receptors). The other combined with adrenaline in higher concentrations, leading to contraction of the muscle (motor receptors). If both types were stimulated, the effect of the motor receptors generally preponderated. Ergotamine and other adrenergic blocking drugs were believed to block the motor receptors more easily than the inhibitor receptors, this explaining the adrenaline reversal after administration of such drugs.

If, however, we seek to explain the effect of adrenaline on the smooth muscle of blood vessels with the aid of this theory, we shall have to make a number of assumptions that are either implausible or directly conflict with facts. The principal objections to the theory that both motor receptors and inhibitor receptors are present in the same cell of vascular muscle are as follows:

1. While intravenous or intra-arterial infusion of adrenaline produces, according to the dose, dilatation or constriction of skeletal muscle vessels, adrenaline in all active concentrations constricts isolated ones (ROTHLIN, 1920; VON TAVEL, 1933). BRUN, on local application of adrenaline to muscle arterioles in rats (1947) and cats (personal communication), was able to demonstrate only a constrictor effect. Dale & RICHARD (1918) assumed that adrenaline, in common with histamine, constricted arteries and arterioles but dilated capillaries. The opposite effects of adrenaline in small and large doses might, therefore, be attributed to different responses of different vascular areas to adrenaline. Yet HARTMAN, EVANS & WALKER (1928), in direct microscopic observation of vessels in the sartorius muscles of ether-anesthetized cats, found that after 2 μg adrenaline intravenously, both arteries and arterioles and capillaries, venules and veins were dilated, whereas after 10 μg only the capillaries were dilated and other vessels constricted. Intravenous administration of adrenaline accordingly dilated in low doses the same vessels that it constricted in higher ones. Since the capillaries of mammals do not possess their own motor system (FOLKOW, 1955), the dilatation following the larger dose of adrenaline may have been merely passive to pressure.

The only isolated vessels that adrenaline certainly relaxes are coronaries from cow and swine (FURCHGOTT, 1955; MOHME-LUNDHOLM, 1956); but these are relaxed even by adrenaline in high concentrations. It follows that either motor receptors or inhibitor receptors may be demonstrated, though not both coincidently, in isolated vessels that have not been pretreated with adrenergic blocking drugs. — Adrenaline elicited its relaxing effect on bovine coronary vessels by inducing lactic acid production in the smooth muscle (MOHME-LUNDHOLM, 1956). The mechanism of the relaxing effect on vascular muscle is thus identical with that on other smooth muscle (MOHME-LUNDHOLM, 1953).

Thus, while intravenous or intra-arterial infusion of adrenaline may, according to the dose, produce dilatation or constriction of vessels in communication with skeletal muscle, the same cannot be done on isolated vessels.

2. In the dog, the sympathetic transmitter substance probably consists chiefly of noradrenaline (EULER, 1951). — On infusion of noradrenaline in low concentrations into the femoral artery, a vasodilator effect was produced (GOTTSTEIN, HILLE & OBER-

DORF, 1955). Small doses of noradrenaline even had a depressor effect when injected intravenously (LANDS, LUDUENA, ANANENKO & GRANT, 1950). In the dog noradrenaline, like adrenaline, thus acts as a vasodilator or a vasoconstrictor according to the dose; this in contrast to the state of affairs in man, cat and rabbit, where noradrenaline does not exert a vasodilator effect until adrenergic blocking drugs have been administered. The receptors with which noradrenaline combines in the smooth muscle are generally assumed to be identical with those with which adrenaline combines (FURCHGOTT, 1955).

Under basal conditions, however, the vessels possess a certain amount of tonus because noradrenaline is released at the sympathetic nerve endings. It must therefore be assumed that, at this noradrenaline concentration, the effect of the motor receptors preponderates. If, however, the noradrenaline concentration is raised somewhat by injection, vasodilatation will follow; *i. e.*, the effect of the inhibitor receptors will preponderate. With a further increase of the noradrenaline concentration the motor receptors will once more have the predominant effect. From this it follows that weak stimulation of the sympathetic nerves in the dog might be expected to have a dilator effect. According to UVNÄS (1954), however, it is probable that no adrenergic vasodilator nerves exist, but that the sympathetic dilators are cholinergic. It should be possible to demonstrate this triphasic effect of noradrenaline on isolated vessels too; but that substance, like adrenaline, relaxes only the coronary vessels in some species of animals, and otherwise has a purely constrictor effect.

3. If adrenaline is administered to isolated coronary vessel, rabbit gut or other smooth muscle organs that are relaxed by adrenaline, the inhibitory effect will persist as long as active adrenaline remains in the solution. This inhibitory effect of adrenaline, which may persist for hours, differs appreciably from the vasodilatation of but a few minutes' duration that is induced by intra-arterial infusion. On the other hand, adrenaline administered intravenously causes dilatation of longer duration. It seems highly improbable that the inhibitor receptors in the smooth muscle of the blood vessels are able to distinguish between intra-arterially and intravenously administered adrenaline.

4. Chloralose anesthesia appreciably inhibited the lactic acid producing effect of adrenaline in skeletal muscle (LUNDHOLM, 1949), and it also inhibited the vasodilator effect (see Part II).

Yet the relaxing effect on isolated coronary vessels and bronchial muscle from cow, and on rabbit gut, was not even inhibited by chloralose in a 1 per cent solution (MOHME-LUNDHOLM, unpublished experiments). It seems most unlikely that chloralose would selectively block the inhibitor receptors in the skeletal muscle vessels but not similar receptors in the coronary vessels and other smooth muscle, and that the coincident blocking of the lactic acid production in skeletal muscle would be an irrelevant factor.

If the vasodilator effect of adrenaline is assumed to be due to lactic acid production in the skeletal muscle cells, while the constrictor effect is localized directly to the smooth muscle layer of the vessels, then the apparently conflicting experimental results will have a simple explanation; and this will also explain the difference in effect between the sympathetic transmitter liberated at the sympathetic nerve endings and the transmitter substance supplied via the blood. On local application of adrenaline directly to the vessel, or liberation of noradrenaline and adrenaline in, or contiguous to, the unstriped muscle of the blood vessels due to sympathetic stimulation, only a vasoconstrictor effect is found. If adrenaline is administered via the arterial blood, the concentration in the tissues will initially be only high enough to accelerate the lactic acid production in the skeletal muscle, the result being relaxation of the vascular muscle. Not until the adrenaline concentration has increased further will it be sufficiently high to overcome the relaxing effect of lactic acid, and in addition the initial tonus of the vascular muscle will rise, since the threshold concentration for the lactic acid producing effect of adrenaline is lower than that for the constrictor effect on skeletal muscle vessels.

In experiments on ether-anesthetized cats the threshold dose for the lactic acid producing effects of adrenaline was 0.1 μg/kg/min with intravenous infusion, but not until the dose had reached 24 μg/kg/min was primary vasoconstriction in skeletal muscle obtained (see Part II). — Similar findings have been reported in man. CORI & BUCHWALD (1930) found that the threshold dose for the lactic acid producing effect of adrenaline, infused intravenously in human subjects, was 0.025—0.05 μg/kg/min; while ALLAN, BARCROFT and EDHOLM (1946) observed that even an intravenous infusion in a dose of 1 μg/kg/min — corresponding to about 0.015 μg/kg/min — increased the blood flow in the

muscles of the forearm. HARPUDER, BYER & STEIN (1947) found the threshold dose of adrenaline for primary vasoconstriction in human skeletal muscle to be 1—2 μg on injection into the brachial artery. However, the vasoconstrictor effect of adrenaline should already have been appreciable at lower doses, since a dose of 0.1—0.5 μg had no effect on the blood flow in the forearm muscles, whereas doses of 0.0002—0.05 μg induced vasodilatation. In a certain area of concentration the vasoconstrictor effect of adrenaline was thus counteracted by the vasodilator action, manifested in the subsidence of vasodilatation with increasing doses.

The cause of the brief dilator effect of adrenaline administered by intra-arterial infusion will be discussed later on, as well as the differences in duration of vasodilatation after intra-arterial and intravenous infusion.

I would point out that this discussion refers to the receptors in the smooth muscle of the vessels. The presence of coincidently functioning inhibitor and motor receptors in other kinds of smooth muscle is not disputed.

Relation Between Blood Flow, Lactic Acid Production and Carbon Dioxide Production.

Under basal conditions lactic acid was continuously produced in the skeletal muscle cells, and the rate of this production could be expected to influence the vascular tonus and hence the blood flow. This, however, did not seem to be the case. When the basal values for blood flow and lactic acid production in the various experiments were compared no correlation was found ($r = 0.07$), even though the lactic acid elimination varied from -0.7 to 17.0 mg/kg muscle/min (Table VI). The same was found to be the case with adrenaline infusion. The blood flow returned to the initial level 10—15 minutes after the start of infusion, although the lactic acid production was still increased. Nor on comparison of the increase in blood flow and that in lactic acid production for the different 5-minute periods in the adrenaline experiments, was any correlation demonstrable ($r = 0.09$). There was, on the other hand, a highly probable correlation between the rise of blood flow and of the CO_2 production ($r = 0.66$, $0.01 > P > 0.001$). This will be seen too from Fig. 3, where the curves for blood flow and CO_2 production run almost parallel.

The elevated CO_2 production could scarcely have been of metabolic origin. In the adrenaline experiments the respiratory quotient

Table VI.

Statistical analysis of the correlations between blood flow and oxygen consumption, carbon dioxide production (ml/kg muscle/min) and lactic acid production (mg/kg muscle/min).

Correlation between:		Number of tests	Coefficient of regression	Coefficient of correlation	P
Basal values	Blood flow and:				
	1. O_2-consumption	22	10.2	0.76	< 0.001
	2. CO_2-production	22	10.6	0.70	< 0.001
	3. Lactic acid production ...	22	0.76	0.07	0.7—0.8
Adrenaline tests	Blood flow and:				
	1. O_2-consumption	20	4.5	0.17	0.4—0.5
	2. CO_2-production	20	9.7	0.63	0.001—0.01
	Increase in blood flow over basal values and increase over basal values of:				
	1. CO_2-production	20	6.4	0.66	0.001—0.01
	2. Lactic acid production ...	20	0.78	0.09	0.7—0.8

was initially 1.0 but rose to 1.74 during the first five minutes of the adrenaline infusion, which rise could hardly have been due to altered metabolism. Nor was it likely to have been a case of carbon dioxide elimination as a result of the increased blood flow, since the CO_2 content of the venous blood was scarcely changed, and hence the CO_2 tension should have been constant too, since the lactic acid content showed no alteration either. The rise in the CO_2 production may have resulted, rather, from the increased lactic acid content of the tissues, whereby CO_2 was discharged from the bicarbonate in the cells and intercellular fluid. GRIFFITH, OMACHI, LOCKWOOD & LOOMIS (1947), who also observed an increase of the CO_2 production in cats' hind legs after infusion of adrenaline, offered a similar explanation.

Since the CO_2 production was apparently the factor that determined the blood flow in the adrenaline experiments, it seemed worth while finding out if that production had any bearing on the volume of the blood flow under basal conditions too. With this aim in view the blood flow and the CO_2 production were compared in all basal experiments (Table VI). A statistically

significant correlation was found. Yet the correlation between oxygen consumption and blood flow was equally evident, as was to be expected when the respiratory quotient was 1. It could not be established, therefore, whether the CO_2 production or the O_2 consumption was the factor primarily responsible. However, when the blood flow was compared with the CO_2 production and O_2 consumption in the adrenaline experiments, where the relation had been calculated for each 5-minute period, it still showed a statistically significant correlation to the CO_2 production, but no longer to the O_2 consumption. Moreover, the correlation between blood flow and CO_2 production was found to be very similar in the basal and the adrenaline experiments. The regression equation for the basal experiments was $Y = 54.5 + + 10.6\ X$, and for the adrenaline experiments $Y = 77.5 + 9.7\ X$, where Y is the blood flow and X the CO_2 production. Analysis of covariance showed that the probability of the two regression equations being identical lay between 0.2 and 0.05. The result of this analysis thus indicated that under basal conditions the CO_2 production was a stimulating factor for the blood flow, and that the effect of adrenaline was to increase that stimulation.

In the adrenaline experiments the total increase of the CO_2 elimination in the blood amounted to 0.12 mM/kg muscle/min, while that of the lactic acid production — blood and muscle — was 0.10 mM. There was accordingly a satisfactory measure of equivalence, though this in itself was somewhat surprising; for we may expect *a priori* to find equivalence between the amount of lactic acid retained in the muscle — 0.06 mM/kg muscle/min — and the rise in the CO_2 elimination. Although the lactic acid that diffused into the blood stream might conceivably have separated CO_2 from the blood bicarbonate, this should not have caused any increase of the CO_2 production in the muscle. The variability in the experiments was too great for the expected value for the CO_2 production to show any statistically probable difference from that obtained experimentally.

D'Silva (1934) observed, however, that adrenaline elevated the potassium concentration in the blood; and Fenn, Koenemann, Favata & Sheridan (1940) showed that the increase in the potassium ions was greater than that in the lactate ions. — It seems probable, therefore, that the lactate ions diffused from the cells together with potassium ions. If so, then equivalence

should be expected between the total lactic acid produced and the rise in the CO_2 production.

The correlation between the increases of blood flow and of the CO_2 production showed that it was only a change in the lactic acid content of the tissues that induced vasodilatation; apparently, no importance attached to the absolute content of lactic acid or to the lactic acid production when equilibrium had been reached between the amounts produced in and diffused from the skeletal muscle cells. That lactic acid leaves the cells by diffusion seems probable, since the mean content in the muscle was 41.4 mg per cent and in venous blood from the muscle, 29.0 mg per cent. Calculation of the lactic acid content in the aqueous phase in the two types of tissue showed a concentration gradient of 20.3 mg per cent between muscle and blood. The water content of muscle has been assumed to be 75 per cent, and of blood 83 per cent (FENN, COBB, MANERY & BLOOR, 1938). However, the diffusion rate of lactate in skeletal muscle is substantially less than that of various gases (EGGLETON, EGGLETON & HILL, 1928). — Hence the first effect that should have been expected from injection of adrenaline was an increase in the CO_2 elimination. This, indeed, was the case. During the first five minutes of the adrenaline infusion, the CO_2 elimination rose by 0.2 mM/kg muscle/min, but the lactic acid elimination by only 0.04 mM. During the second 5-minute period the corresponding values were 0.13 and 0.03 mM respectively. Only after 10—15 minutes infusion did the lactic acid elimination in the blood show an appreciable rise — 0.07 mM — and at the same time exceeded the CO_2 elimination, which had fallen to — 0.07 mM/kg muscle/min. The fall in the CO_2 elimination was due to a decrease of the oxygen consumption under the influence of adrenaline. Coincident with the maximum lactic acid elimination the blood flow had returned to the initial level, this result being consistent with that reported by GOLLWITZER-MEIER and FACK (1949). Those authors, in experiments on the hind legs of cats, observed that the blood pH fell during intra-arterial infusion of adrenaline, even in cases where the lactic acid elimination was initially unchanged or had decreased. Not until the infusion had ended did the lactic acid elimination increase, concurrently with a rise in the pH. The same result was found regardless of whether the blood flow rose or fell.

We thus obtain the following picture of the action of adrenaline.

Under basal conditions there is equilibrium between the amount of lactic acid produced and the amount leaving the cells by diffusion into the blood. On administration of adrenaline the rate of lactic acid production increases two- or threefold. The diffusion gradient between tissues and blood will be too small for the produced lactic acid to be eliminated by diffusion, and the lactic acid content and acidity will rise in the tissues. As a result of the increased acidity in the skeletal muscle cells, these latter will discharge carbonic acid and carbon dioxide into the extracellular fluid, the pH of which will fall, leading to vasodilatation and elevation of the blood flow, so that the diffusion gradient for lactic acid will increase. (*Cf.* the experiments on cats' hind legs, in which the lactic acid content of the venous blood decreased when adrenaline was infused.) The increased blood flow will also accelerate the CO_2 elimination, so that the acidity will tend to diminish in the tissues, but with it the stimulus to vasodilatation will decrease too. There will then be a tendency for the blood flow to fall; but in so doing, the diffusion gradient for lactic acid will decrease and the latter's content will rise in the tissues, thus counteracting in some degree the first-named tendency.

In this way there will be interaction between those factors which tend to increase, and those which tend to decrease, the vasodilatation, until a new state of equilibrium is reached in which the amount of lactic acid produced in the cells and that diffused from the cells will again be alike. The diffusion gradient will have increased because the lactic acid content of the tissues has risen, but since this has occurred at the cost of the bicarbonate ions, there will be only a very slight fall in the tissue pH. — In my experiments the new state of equilibrium that gradually appeared was distinctly reflected in the progressive diminution of the blood flow (Fig. 1 A).

In the four experiments where the blood flow returned to its initial level after 15 minutes' infusion of adrenaline, the diffusion gradient between venous blood and muscle had increased from 15.1 before the infusion to 27.8 mg per cent at its completion; *i.e.*, by 84 per cent. The lactic acid elimination in the blood had then risen by 98 per cent; *i.e.*, there was a linear relation between the diffusion gradient and the elimination of lactic acid, in agreement with the result reported by Eggleton, Eggleton & Hill (1928).

The lactate ions may have little or no influence on the pH of the extracellular fluid, since they probably diffuse out of the muscle cells together with potassium ions.

On intra-arterial infusion of adrenaline in a small vascular area the lactic acid content of the arterial blood remains constant, and the new state of equilibrium between produced and diffused lactic acid should be reached fairly rapidly, so that the stimulus to vasodilatation will disappear. With intravenous infusion, however, we find a different state of affairs. With the rise in lactic acid production throughout the skeletal muscle, the lactic acid content will progressively increase in the arterial blood. A new state of equilibrium between produced and diffused lactic acid will not be attained until the lactic acid content of the arterial blood has become constant, this taking about 40 minutes (see Part II). Throughout that time the lactic acid content of the tissues should be rising, and hence an intravenous infusion of adrenaline should have a more prolonged vasodilator effect than an intra-arterial infusion in a small vascular area.

The explanation of a few other peculiar results after intra-arterial infusion of adrenaline may be that it is only a rise in the lactic acid content of the tissues that is attended by vasodilatation. Barcroft & Swan (1953) reported that with continuous infusion of adrenaline into the human brachial artery, and a rise step by step of the adrenaline concentration, the blood flow increased for a short time whenever the dose of adrenaline was raised. A constant increase of the blood flow was not possible to obtain. — I was able to verify these results in similar experiments on cats. The explanation is probably that the adrenaline concentration and the rate of lactic acid production are in some degree commensurate. (Part II.) Each rise in the adrenaline concentration was associated with a corresponding increase in the rate of lactic acid production. The blood flow increased whenever the lactic acid concentration rose in the tissues.

With repeated injections of adrenaline the amplitude and duration of vasodilatation, as described earlier, fell. Similar findings were reported by Bell & Stead (1952). Yet if the injections were given at sufficiently long intervals — 15—30 minutes — it was generally possible to induce equal degrees of vasodilatation by repeated infusions of short duration. The explanation may well be that some time elapses before the lactic acid content of the tissues has returned to the initial level.

Until this has taken place the lactic acid content will, on further infusion of adrenaline, show a comparatively lesser increase, and hence the vasodilatation too will be less.

In this connection the mechanism of the dilator effect of lactate ions will also be discussed. Under basal conditions an intra-arterial injection of sodium lactate in the amounts produced at adrenaline administration, induced little or no vasodilatation; nor did the lactate ion have any dilator effect on unanesthetized animals (Part III). The absence of any vasodilator effect of the lactate ion under basal conditions was also evident from the fact that no correlation existed between the basal lactic acid production and the blood flow, and that although the latter production rose during infusion of adrenaline, the blood flow gradually returned to the initial level. During infusion of adrenaline, on the other hand, administration of lactate ions either intra-arterially or intravenously had an appreciable dilator effect as in deeply anesthetized animals, in which the lactic acid production was increased. With an increase in the lactic acid content of the arterial blood, the diffusion gradient for lactate ions between tissues and blood should initially decrease and the lactic acid content should rise in the muscles, the result being increased acidity. But if the lactic acid production is elevated, as in adrenaline infusion, then with an abrupt fall in the diffusion gradient lactic acid should accumulate faster in the muscles than it does with a lower lactic acid production; the acidity, too, should increase more rapidly, with greater vasodilatation as the result. However, the absolute increase of lactic acid in the muscles, when equilibrium has again been reached, should be the same at a low and a high lactic acid production. For the amount of lactic acid diffused through any given tissue is directly proportional to the diffusion gradient (Eggleton, Eggleton & Hill, 1928).

If the lactic acid content rises in the tissues, then CO_2 should be expelled from bicarbonate, so that the CO_2 elimination will increase. This, indeed, seems to be the case, as will be seen from Fig. 4, where the carbon dioxide production rose during the first five minutes of lactic acid infusion. Superficially, one might perhaps be inclined to attribute this effect to the hydrogen ion in the infused lactic acid, but further analysis shows that it can scarcely be capable of elevating the CO_2 production. On intra-arterial infusion of lactic acid the lactate ion concentration rose

by about 1.1 mM per liter (10 mg per cent), whereas the hydrogen ion concentration, due to the buffering ability of the blood, could only have increased $5 \cdot 10^{-6}$ mM per liter (from pH 7.40 to pH 7.35); *i.e.*, the concentration of lactate ions rose two hundred thousand times more than that of the hydrogen ions. Under such conditions equivalent amounts of lactate ions and hydrogen ions cannot be expected to diffuse into the muscles. If diffusion of lactate ions to the muscles does occur, then it is probably together with potassium or sodium ions. Yet in so doing, the CO_2 production cannot be expected to increase, since it would presuppose the expulsion of CO_2 from the *tissue* bicarbonate. With the method employed here for determining the CO_2 production, a decrease in the bicarbonate content of the blood through addition of lactic acid did not cause any rise in the CO_2 elimination.

The lactic acid content of the muscles increased, however, by an average of 5.4 mg/kg muscle/min during infusion of lactic acid. This increase was probably due, not to diffusion of lactate ions from the blood but rather to a decreased diffusion of lactic acid from the muscles. On intra-arterial infusion of lactic acid, the latter's content in the arterial blood rose by an average of 10 mg per cent. Under basal conditions the lactic acid content of the arterial blood was 19.3 mg per cent. Assuming the hematocrit value to be 35 per cent, the water content of the blood cells 65 per cent and of the plasma 92 per cent (Fenn, Cobb, Manery & Bloor, 1938), the water content of whole blood was 83 per cent and the lactic acid concentration in the aqueous phase 23 mg per cent. — When lactic acid is added to whole blood it is chiefly distributed in the aqueous phase of the plasma, since the penetration into the blood cells is incomplete (Devadetta, 1934). — The total lactic acid concentration in the aqueous phase of plasma may therefore be estimated to have been not more than 40 mg per cent. The lactic acid content of the control muscle was 39.3 mg per cent, and since the water content was calculated at 75 per cent the lactic acid concentration in the muscle's aqueous phase amounted to 52 mg per cent. The lactic acid content of the aqueous phase of blood thus did not reach, even at the start of infusion, the same level as that in the muscles; and from this it may be deduced that the elevated lactic acid content of the muscles was due to reduced diffusion from the skeletal muscle cells, and not a diffusion from the blood to the muscles. This assumption is supported, moreover, by the

fact that the mean increase of lactic acid in the muscles (5.4 mg/kg muscle/min) approximated the lactic acid elimination in the blood under basal conditions (6.2 mg/kg muscle/min). — But if lactic acid accumulates in the muscle cells, then it should liberate CO_2 from bicarbonate, thus increasing the CO_2 production. — In the lactic acid experiments, some rise in the CO_2 production was noted during the first 5-minute period (Fig. 4), but the increase as a whole was not statistically verifiable. With a mean rise of 0.06 mM/kg muscle/min (5.4 mg/kg muscle/min) in the lactic acid content of the muscles, the CO_2 production should have risen to an equal degree; but in fact the increase was only 0.007 mM after a correction had been made for the rise in the O_2 consumption. It could be expected, on the other hand, that as a result of the increased CO_2 tension in the blood through the addition of lactic acid, the diffusion gradient for CO_2 between tissues and blood would fall, and hence carbon dioxide should accumulate in the tissues and the CO_2 elimination decrease. Since the latter nevertheless rose, the implication is that there was a real increase of the CO_2 production.

It is evident from the foregoing that intra-arterial infusion of lactic acid elevates the acidity in the tissues in two ways: by the supply of hydrogen ions and by hindering the diffusion of the lactic acid produced under basal conditions. This explains why lactic acid had a more powerful vasodilator effect than an equivalent amount of hydrochloric acid.

It seems clear from the above that the lactate ion may be expected to have a vasodilator effect only when there is a continuous production of lactic acid in the tissues. The lactic acid production, as mentioned earlier, seems to be elevated in ether-anesthetized cats by comparison with that in unanesthetized animals. The lactate ion, accordingly, did not seem to have any specific vasodilator effect in unanesthetized rabbits (Part III). The lactate ion, moreover, might be expected to have a dilator effect only in such vascular areas as skeletal muscle, where lactic acid is continuously produced.

Summary

The mechanism of the vasodilator effect of adrenaline in skeletal muscle was investigated in cats anesthetized with ether. Adrenaline was administered by intra-arterial infusion.

The vasodilator effect was regularly found only after appropriate doses of adrenaline and relatively atraumatic preparation. If the animal was in poor condition, too deeply anesthetized, or hyperthermal, or if the infused vascular area had been cooled, only the vasoconstrictor and not the vasodilator effect of adrenaline appeared. The vasodilatation was most pronounced and of longest duration under ether anesthesia, but emerged distinctly under urethane anesthesia too; under evipan and chloralose anesthesia it was weak and of short duration.

Continuous infusion of adrenaline into the brachial artery elevated the blood flow up to double or treble. The increased blood flow persisted for an average of 10 minutes, but then disappeared in spite of continued infusion of adrenaline.

The lactic acid production, CO_2 production and O_2 consumption of the muscles were calculated from the values for the arteriovenous differences and the blood flow. For quantitative determination of the lactic acid production it was also necessary to determine the changes in the lactic acid content of the muscles. The changes in lactic acid production were reflected incompletely and with some delay in the venous blood.

During infusion of adrenaline the lactic acid production rose two- to threefold and, taking the mean of the eight experiments, by 9.4 mg/kg muscle/minute. The CO_2 elimination rose too; the increase in blood flow and in CO_2 production showed fully parallel courses, and the elevated lactic acid production still persisted when the blood flow had returned to the initial level. The oxygen consumption fell by 16 per cent.

On intra-arterial infusion of lactic acid in an amount equivalent to that produced during infusion of adrenaline, there was an increase in blood flow, the magnitude and general course of which were identical with those after adrenaline infusion.

Substances that blocked the lactic acid production, such as copper ions and sodium fluoride, completely inhibited the vasodilator effect of adrenaline and changed it to constriction.

Intra-arterial or intravenous infusion of adrenaline induced vasodilatation or vasoconstriction, depending on the concentration. In vessels isolated from skeletal muscle, adrenaline induced constriction alone in all active concentrations. Earlier experiments which suggested that some vessels — renal arteries — were relaxed by adrenaline in low concentrations but constricted in higher concentrations, were found to have been misinterpreted.

— The vessels in question possess both a circular and a longitudinal muscle layer. — On contraction of the longitudinal muscle fibers there was a shortening of the circular layer, presenting as relaxation. The notion is therefore rejected that the smooth muscle of the vessels has coincidently functioning inhibitor and motor receptors.

The vasodilator effect of adrenaline is assumed to be due, rather, to the lactic acid production in the skeletal muscle cells, while the constrictor effect is localized to the smooth muscle layer of the vessels. — The threshold concentration for the lactic acid producing effect was substantially lower than that for the vasoconstrictor effect.

The vasodilator action of adrenaline occurred only during an increase in the lactic acid content of the muscle. The absolute magnitude of the lactic acid production, on the other hand, was of no significance. When equilibrium had been reached between produced and diffused lactic acid, the vasodilatation subsided notwithstanding continued infusion of adrenaline. If diffusion of lactic acid from the tissues was prevented by intravenous or intra-arterial infusion of sodium lactate, the dilator effect of adrenaline was amplified or prolonged because of lactic acid accumulation in the tissues.

The increase in blood flow showed a statistically significant correlation to the rise in CO_2 elimination, but there was no significant correlation between the increased blood flow and the lactic acid elimination in the blood. However, the total amount of lactic acid produced was matched by an equivalent increase in the CO_2 production. — Hence, only a minor part of the vasodilator effect of adrenaline seems to be a direct action of lactic acid on the vessels. Probably the increased acidity of the extracellular fluid was largely a result of the rise in the carbonic acid content and CO_2 tension that occurred in the tissues when the lactic acid was buffered with the alkali conjugated to the muscle cell bicarbonate.

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